SEQ ID NO:19 GGAATGACGC AAGGACATAT GGGCGT,
SEQ ID NO:20 CCCAGGTGCA CACCAATGTG GTGGAT,
SEQ ID NO:21 GGACTGTGCG CGTTGTATAC CCTGC,
SEQ ID NO:22 ACTCCCGAAG CGAATGGCAC GTGGA,
SEQ ID NO:23 CATAGCTTGT GCCCGTGTGG CACGT,
SEQ ID NO:24 CCAAGACGAG ACCGTCAGAG CTGGT,
SEQ ID NO:25 AAGCTGTTGC CGCCATCAAA TAAACG, [or] and
SEQ ID NO:26 CTGCGTTAGA CCGAGAACTG TGGATAAAGG.

C1

Please add the following new claims 39-42:

39. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

C2

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said

first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3', and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

(c) a thermostable DNA polymerase present at at least 10 units/100 µl.

C2

40. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each

other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

a thermostable DNA polymerase present at at least 10 units/100 μl,

b) at least one additional PCR reagent, and

c) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

41. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

C2

each of said first and second primers being present in the same amount within the range of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said primer T_m's being within about 5°C of each other, [and]

said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3', and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3',

b) a separate aqueous composition buffered to a pH of from about 7 to about 9, and comprising third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second

target DNA which is the same as or different from hCMV DNA and which are separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said third and fourth primers being present in the same amount of from about 0.1 to about 2 μmolar and having a T_m within the range of from about 65 to about 74°C, said third and fourth primer T_m's being within about 5°C of each other and within about 5°C of the T_m's of said first and second primers, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides,

c) included in either a) or b), a thermostable DNA polymerase present at at least 10 units/100 μl,

C2

d) at least one additional PCR reagent, and

e) a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

42. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

C2

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and each of said first, second, third and fourth primers being present in the same amount within the range of from about 0.1 to about 2 μmolar, and

said first and second primers being SEQ ID NO:1 and SEQ ID NO:2, respectively, in Primer set 1, or said first and second primers being SEQ ID NO:3 and SEQ ID

NO:4, respectively, in Primer set 2, identified as follows:

Primer set 1:

SEQ ID NO:1 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

SEQ ID NO:2 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

SEQ ID NO:3 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

SEQ ID NO:4 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

ii) the following additional PCR reagents: a thermostable DNA polymerase present in an amount of at least 10 units/100 µl, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

C2 to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C and carried out within 120 seconds,

B) capturing one of said amplified hCMV DNA strands with a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

capturing one of said amplified second target DNA strands with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C, and

wherein said first capture probe is selected from the group consisting of:

SEQ ID NO:5 5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

SEQ ID NO:6 5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

SEQ ID NO:7 5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3',

C2

and

SEQ ID NO:8 5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3',

and

C) simultaneously detecting said captured amplified hCMV DNA strand and said captured amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and said second target DNA.

REMARKS

This is the second Rule 116 amendment presented for this application. In her Advisory Action mailed July 19, 1994, the Examiner indicated that the first Rule 116 Amendment would not be entered because of certain alleged defects in the language of new Claims 39-42. It is believed that these alleged defects have been corrected with the foregoing amendment. Specifically, the first and second primers are defined more particularly by Sequence Listing ID No's for the recited primer sets. These primers are the subject of cancelled Claim 9. Thus, they have been searched and considered previously.

In addition, it is noted from MPEP 607, that Applicants paid for new independent claims 39-42 with their earlier Rule 116 Amendment, and thus, Applicants need not count them in calculating any additional fees with this amendment.

The Advisory Action also indicated that the previous amendment, if entered would have overcome the objection to the drawings and specification, and removed the rejection of Claim 38 under Section 112(2). It is believed that the present amendment has the same effect. The corrected drawings were submitted earlier, so they are not being submitted again. The remaining remarks